

Contents lists available at ScienceDirect

## Journal of Asia-Pacific Entomology



journal homepage: www.elsevier.com/locate/jape

# Temperature-dependent development of Acarophagous ladybird, Stethorus gilvifrons (Mulsant) (Coleoptera: Coccinellidae)

## Roya Taghizadeh, Yaghoub Fathipour \*, Karim Kamali

Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, P.O. Box: 14115-336, Tehran, Iran

## ARTICLE INFO

Article history: Received 18 May 2008 Revised 30 June 2008 Accepted 1 July 2008

Keywords: Acarophagous ladybird Degree-day Development rate Temperature Two spotted spider mite

## ABSTRACT

Development of Stethorus gilvifrons (Mulsant), a predator of two spotted spider mite (Tetranychus urticae Koch), was studied under laboratory conditions at constant temperatures of 15, 20, 25, 28, 30, 35 and 40°C. No development occurred at 40°C. The total development time at temperatures tested was 56.47, 31.19, 18.53, 17.54, 12.49, and 9.27days, respectively, which indicated a significant decrease of development time with increasing temperature. Using the linear model, the estimated low temperature threshold for egg, larva, prepupa, pupa, and total immature stage of *S. gilvifrons* was 14.11, 10.86, 11.33, 17.04, and 12.47°C, respectively while the thermal constant for these stages was 36.36, 153.14, 11.63, 25.25, and 222.72 degree-days, respectively.

© Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society, 2008. Published by Elsevier B.V. All rights reserved.

al., 1993; Felland and Hull, 1996; Roy et al., 1999).

concentrated on the efficacy of Stethorus spp. in orchards (Congdon et

mite and insect pests and their natural enemies (Huffaker et al., 1999).

Temperature sets the limit of biological activities in arthropods, such

that low and high temperature thresholds and optimal temperature

can be estimated for all major life processes (Roy et al., 2002). Thermal

characteristics may vary between species and populations (Lee and

Elliott, 1998) and with other ecological factors such as food source

(Gilbert and Raworth, 1998). Development rate, expressed as the

reciprocal of development time needed to change from one stage to

another, is near zero at the low temperature threshold, increases with

temperature and levels off at the optimum, and then decreases rapidly

as the high threshold is approached. This relationship is curvilinear

near the extremes, but approximately linear at moderate tempera-

Temperature is a critical abiotic factor influencing the dynamics of

## Introduction

Tetranvchus urticae Koch is a polyphagous and parenchyma cellfeeding pest on a variety of annual and perennial crops (Han et al., 2003; Jung, 2005; Jung et al., 2005) with over 200 host plant species (Kheradpir et al., 2007). Integrating biological control with chemicals in the spider mite management programs is particularly attractive because biological control of these pests has been implemented successfully on various horticultural crops (Helle and Sabelis, 1985). Two important groups of spider mite predators are predatory mites (Acarina: Phytoseiidae) and coccinellid beetles of the genus Stethorus (Coleoptera: Coccinellidae) (Roy et al., 2005). All known species of the genus Stethorus are predators of spider mites (McMurtry et al., 1970; Felland and Hull, 1996) and several species have been reported as regulatory agents of tetranychid pests in agricultural systems (Roy et al., 1999, 2005).

Stethorus gilvifrons is an important predator of spider mites, such as T. urticae, in Iran and many other neighboring countries (Hajizadeh et al., 1992; Afshari et al., 2001). As specialist predators, Stethorus spp. can effectively control spider mite populations, as shown for S. punctillum (Weise) on cotton and vineyards in Europe (Kapur, 1948) and citrus in China (Yang et al., 1996), and S. punctum LeConte on apple in the USA (Hull et al., 1977). Most studies, however, have been

tures (Wagner et al., 1984). A variety of models have been proposed to describe the relationship between temperature and arthropod development (Logan et al., 1976; Wagner et al., 1984; Lamb, 1992; Lactin et al., 1995: Briere et al., 1999). Temperature-driven models are most often used to predict the activity and seasonal population

1992). The objective of this study is to determine the thermal development characteristics of S. gilvifrons populations. These characteristics are used in forecast models to predict population levels and to determine thermal adaptation of this predator, which will help elucidate the optimal conditions for control of T. urticae in IPM programs.

dynamics of pests and natural enemies in field situations (Lamb,

Corresponding author. Fax: +98 21 44196524. E-mail address: fathi@modares.ac.ir (Y. Fathipour).

<sup>1226-8615/\$ -</sup> see front matter © Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society, 2008. Published by Elsevier B.V. All rights reserved.

## Materials and methods

#### Mite and predator cultures

The spider mite, *T. urticae* and its predator, *S. gilvifrons* were originally collected from the Varamin region in the vicinity of Tehran, Iran on bean leaves in 2005. The specimens were transferred to the laboratory and used for the experiments. *Tetranychus urticae* was reared on bean leaves (*Phaseolus vulgaris* L. cv. Sunray) in a growth chamber at  $25^{\circ}$ C,  $65 \pm 5\%$  R.H. and a photoperiod of 16: 8h (L: D). The predatory beetles were reared on bean leaves containing spider mites in the laboratory at the above-mentioned conditions. Water was provided using a moistened dental wick. Seedlings of *P. vulgaris* were grown to the three- to four-leaf stage in the 50:50 ratio of sand clay and peat moss in 100-cm<sup>3</sup> pots.

### Experimental protocol

To obtain egg cohorts, several coccinellid females and males were incubated at 25°C on 4-cm diameter bean leaf discs which were placed in 6-cm diameter Petri dishes. Newly laid eggs of *S. gilvifrons* were then transferred individually on new leaf discs and placed in growth chambers programmed at temperatures of 15, 20, 25, 28, 30, 35, and 40°C, 65  $\pm$  5% R.H. and a photoperiod of 16: 8h (L:D). We used 60 eggs for each temperature. Survivorship and life stage of all individuals were checked and recorded daily. The different larval instars of the predator were determined by their size and larval exuviae. The larvae (four instars) of *S. gilvifrons* were fed daily with *T. urticae* (about 300 preys offered daily). Immature individuals were transferred on fresh leaf discs every two or three days, until coccinellids matured. Development time of immature individuals was determined for the above-mentioned temperatures.

The mean development rate of immature stages of *S. gilvifrons* at different temperatures was calculated using the following equation:

$$DR(T) = 1/EXP[\Sigma \ln(DT)/N]$$
(1)

where DR(T) is the mean development rate at temperature  $T(^{\circ}C)$ , DT is the individual observation of development time in days, and N is the number of observations. This method was recommended by Logan et al. (1976) to account for linearity in the transformation of development time to development rate.

Development rate is the reciprocal of development time in days and it is represented by values from 0 to 1. These rates are used in development models where data are added each day (Medeiros et al., 2004). The development of an organism is completed when the sum of their daily rate of development reaches a value of 1 (Curry and Feldman, 1987). Therefore, the integral of the function of development rate along time can be used to simulate the development of an organism subjected to changes in temperature. For this reason, linear procedure has been used to analyze the relationship between development rate of S. gilvifrons and temperature. Linear regression model, DR(T) = a + bT, where *a* is the intercept and *b* the slope of the regression line, DR(T) and T are the same as in the abovementioned equation, was used to estimate the low temperature threshold and the thermal constant (K) for egg, larva and whole immature stage of S. gilvifrons. The lower temperature threshold for development was calculated as  $T_{min}=-a/b$ , and the thermal constant was determined as K = 1/b. We used the adjusted coefficient of determination  $(R^2_{adj})$  and Akaike index criterion (AIC) (Akaike, 1974) to show fitting rate of data to the linear model at different temperatures. The formulae of the mentioned statistics are as follows:

$$R^2_{adj} = 1 - \left(\frac{n-1}{n-p}\right) \left(1 - R^2\right)$$

where *n* is the number of observation, *p* is the number of model parameters and  $R^2$  is the coefficient of determination;

and AIC = 
$$nLn\left(\frac{SSE}{n}\right) + 2p$$

where SSE is the sum of square of residual error. If  $R^2_{adj}$  be up and AIC be low, which provide data on goodness-of-fit.

#### Statistical analysis

The effect of temperature on the development time of *S. gilvifrons* was analyzed using one-way ANOVA. If significant differences were detected, multiple comparisons were made using the LSD test (P < 0.05). The temperature-dependent model was analyzed using the SPSS statistical program (SPSS, 2004) and thermal constant and temperature threshold were estimated.

#### Results

#### Development time

Stethorus gilvifrons successfully developed to the adult stage from 15 to 35°C. At 40°C no development occurred and egg hatching failed. At all temperatures (15–35°C), mortality rate in egg stage was higher than in subsequent stages. Total development time (oviposition to adult emergence) of S. gilvifrons at 15, 20, 25, 28, 30, and 35°C was 56.47, 31.19, 18.53, 17.54, 12.49, and 9.27 days, respectively. Development time was inversely related to temperature and total development time decreased significantly with increasing temperature between 15 and 35°C. At 15°C, egg development time of S. gilvifrons lasted 13days. This period of time decreased by nearly half at 20°C (6.68days) and then further at 25°C (3.61days). Egg development was completed after 3 and 2.5days at 28 and 30°C, respectively. Total egg development time was the shortest at 35°C (1.5days) (Table 1). Mean development time of various immature stages (egg, total larval period, prepupa, pupa and total development time) of S. gilvifrons at 6 constant temperatures are shown in Table 1. The longest and shortest period for each immature stage was observed at 15 and 35°C, respectively (Table 1).

#### Model evaluation

Using linear regression, the low temperature threshold and thermal constant of different immature stages of *S. gilvifrons* were estimated (Table 2). The development rate for *S. gilvifrons* increased linearly within the examined temperature range (15–35°C). The thermal constant for the complete development period estimated by the linear model was 222.72 DD. The results of parameter estimation of the linear model for development rate of *S. gilvifrons* are presented in Table 3.

#### Discussion

This study was the first to evaluate the effect of temperature on development of *S. gilvifrons* and the results of this research present new information on development and thermal characteristics of this predator. Our findings on seasonal activity and population dynamics, demography, foraging behavior and feeding rate of *S. gilvifrons* revealed that this predator is a good candidate for utilization as a biological control agent for *T. urticae* in both field and greenhouse conditions (unpublished data). For these reasons, estimating temperature-dependent development and thermal requirements using thermal models for *S. gilvifrons* is important for forecast models and understanding its thermal adaptation and synchrony (in preypredator interaction).

#### Table 1

Development time (M±SE) of immature stages of S. gilvifrons at different constant temperatures on T. urticae

Temperature (°C)	Egg	Larva	Prepupa	Рира	Total
15	13.00 ± 1.53 <sup>a</sup>	L(1-4) 29.74 ± 3.07 <sup>a</sup>	$2.00 \pm 0.18^{a}$	11.73 ± 1.17 <sup>a</sup>	$56.47 \pm 3.60^{a}$
		L1 3.07 ± 0.37			
		L2 5.75 ± 0.41			
		L3 10.58 ± 0.58			
		L4 10.33 ± 0.47			
20	$6.68 \pm 1.03^{b}$	L(1-4) 17.76 ± 1.23 <sup>b</sup>	$1.75 \pm 0.18^{b}$	$5.00 \pm 0.58^{b}$	31.19 ± 1.12 <sup>b</sup>
		L1 3.93 ± 0.25			
		L2 4.29 ± 0.22			
		L3 5.08 ± 0.24			
		L4 4.46 ± 0.29			
25	$3.61 \pm 0.48^{\circ}$	$L(1-4) 10.75 \pm 1.30^{\circ}$	$1.00 \pm 0.00^{\circ}$	$3.17 \pm 0.34^{\circ}$	18.53 ± 1.70 <sup>c</sup>
		L1 2.23 ± 0.16			
		L2 3.37 ± 0.20			
		L3 2.00 ± 0.18			
		L4 3.14 ± 0.21			
28	$3.00 \pm 0.63^{cd}$	L(1-4) 10.69 ± 1.01 <sup>c</sup>	$0.85 \pm 0.09^{\circ}$	$3.00 \pm 0.32^{\circ}$	17.54 ± 1.50 <sup>c</sup>
		L1 2.26 ± 0.17			
		L2 3.00 ± 0.16			
		L3 2.50 ± 0.20			
		L4 2.92 ± 0.18			
30	$2.55 \pm 0.4^{\rm d}$	L(1-4) 7.24 ± 0.80 <sup>d</sup>	$0.48 \pm 0.08^{\rm d}$	$2.22 \pm 0.27^{cd}$	12.49 ± 1.43 <sup>d</sup>
		L1 1.81 ± 0.14			
		L2 2.20 ± 0.20			
		L3 1.64 ± 0.13			
		L4 1.58 ± 0.19			
35	$1.50 \pm 0.5^{\rm e}$	$L(1-4) 6.11 \pm 0.76^{d}$	$0.45 \pm 0.04^{\rm d}$	$1.21 \pm 0.22^{d}$	9.27 ± 1.02 <sup>e</sup>
		L1 1.29 ± 0.11			
		L2 1.37 ± 0.12			
		L3 1.44 ± 0.13			
		L4 2.00 ± 0.00			
		L3 1.44 ± 0.13 L4 2.00 ± 0.00			

The means followed by different letters within the same column are significantly different (P < 0.05; LSD). L1, L2, L3, L4 and L(1–4) are first, second, third, and fourth instars, and total larval development times, respectively.

No development occurred at 40 °C.

In this study, development occurred at all temperatures except 40°C, showing that 40°C falls outside the temperature range for development of *S. gilvifrons*. Development rate significantly increased with increasing temperature from 15 to 35°C. The highest development rate was observed at 35°C, which shows this predator is a thermophile species.

The estimation of temperature thresholds and degree-days (DD) for development of natural enemies can substantially contribute to the selection of the most suitable natural enemy to be used at different environmental conditions (Perdikis and Lykouressis, 2002). The lower temperature threshold and thermal constant have been estimated by linear models in numerous studies (Roy et al., 2002). The linear model has been used widely, because it requires minimal data for formulation, is very easy to calculate and apply, and usually yields approximately correct value with negligible differences in accuracy compared to more complex models. Moreover, it is the simplest and easiest method for estimation of thermal constant (K) (Worner, 1992). It should be considered that the linear equation is the only equation that enables the calculation of the thermal constant.

#### Table 2

The low temperature threshold  $(T_{\min})$  and constant thermal (K) of each immature stage of *S. gilvifrons* obtained from linear model

Stage	$T_{\min}(^{\circ}C)$	K(DD)
Egg	14.11	36.36
Larva 1	5.20	44.05
Larva 2	11.03	39.68
Larva 3	12.46	31.54
Larva 4	10.57	43.48
Entire larval period	10.86	153.14
Prepupa	11.33	11.63
Pupa	17.04	25.25
Entire development period	12.47	222.72

From an extensive review of the literature, we found studies on temperature-dependent development of other species of the genus Stethorus. The effects of temperature on the development of S. punctillum (Roy et al., 2002), S. madecassus Chazeau (Chazeau, 1974), and S. loxtoni Britton and Lee (Richardson, 1977) have been investigated. In the present study, the complete development time of S. gilvifrons decreased from 56.47 days at 15°C to 9.27 days at 35°C. The complete development time of *S. punctillum* at 14 and 34°C was 68.5 and 12.1 days, respectively (Roy et al., 2002), demonstrating a slight difference between the two species. Roy et al. (2002) reported that S. punctillum successfully developed between 14 and 34°C on Tetranychus mcdanieli but egg hatching at 12 and 36°C failed. Putman (1955) suggested that S. punctillum had no development at temperatures of < 14 and  $> 36^{\circ}$ C. According to our results, the complete development time of S. gilvifrons on T. urticae at 25°C was 18.53days, whereas Roy et al. (2002) determined this parameter for S. punctillum on T. mcdanieli at 24°C to be 17.1 days, and Shih et al. (1991) for S. loi on T. kanzawai at 23.8°C to be 15.27days. Using linear model, the low temperature threshold of S. gilvifrons was estimated to be 12.47°C; Roy

Table	3
-------	---

Parameters estimated by linear model for describing entire development of S. gilvifrons

Estimates	Parameters	Model
-0.056	а	
0.0045	b	
12.47	T <sub>min</sub>	Linear
222.72	Κ	DR = a + bT
0.951	$R^2$	
0.0003	RSS	
0.939	$R^2_{adj}$	
-66.403	AIC	

DR = Development rate (1/Development time); *a* = Intercept; *b* = Slope; *T* = Temperature.

et al. (2002) determined 12°C as the lower temperature threshold of *S. punctillum*, demonstrating a similar low temperature threshold in both species. The differences between the results of different studies may be due to predator species and experimental conditions. All the mentioned studies demonstrated the significant effect of temperature on development of *Stethorus* spp.

In summary, this study described the development rate and temperature relationships of an Iranian population of *S. gilvifrons* under the range of temperatures generally prevailing in the Tehran region and estimated their key bioclimatic parameters. The findings of this study can be used for predicting of *S. gilvifrons* population development in field conditions. Also, they can be incorporated in models that take into consideration additional information on predation rate, mass rearing, thermal adaptation, synchronic status (prey–predator interactions) for better planning of the usage of this predator for the control of *T. urticae* in both field and greenhouse conditions.

#### Acknowledgments

This research was partly supported by a grant (No. 84-63) from Iran National Science Foundation and partly from Tarbiat Modares University, which is greatly appreciated.

### References

- Afshari, M., Mossadegh, S., Kamali, K., 2001. Feeding behaviors of the ladybird beetle, *Stethorus gilvifrons* (Mulsant), and effect of different factors on its feeding rate in laboratory condition. Sci. J. Agric. 23, 70–90.
- Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control AC. 19, 716–723.
- Briere, J.F., Pracros, P., LeRoux, A.Y., Pierre, J.S., 1999. A novel rate model of temperaturedependent for arthropods. Environ. Entomol. 28, 22–29.
- Chazeau, J., 1974. Evaluation de l action predatrice de Stethorus madecassus (Coleoptera: Coccinellidae) sur Tetranychus neocaledonicus (Acarien: Tetranychidae). Entomophaga. 19, 183–193.
- Congdon, B.D., C.H., Shanks, J.R., Antonelli, A.L., 1993. Population interactions between Stethorus punctum picipes. (Coleoptera: Coccinellidae) and Tetranychus urticae (Acari: Tetranychidae) in red raspberries at low predator and prey densities. Environ. Entomol. 22, 1302–1307.
- Curry, G.L., Feldman, R.M., 1987. Mathematical Foundations of Population Dynamics. TEES Monograph Series, College Station, Texas, p. 249.
- Felland, C.M., Hull, L.A., 1996. Overwintering of Stethorus punctum punctum (Coleoptera: Coccinellidae) in apple orchards ground cover. Environ. Entomol. 25, 972–976.
- Gilbert, N., Raworth, D.A., 1998. Insects and temperature, a general theory. Can. Entomol. 128, 1–13.
- Hajizadeh, J., Kamali, K., Mossadegh, M.S., 1992. Studies on the feeding behavior of Stethorus gilvifrons Mulsant (Col.: Coccinellidae). Sci. J. Agric. 18, 71–88.
- Han, S.H., Jung, C., Lee, J.-H., 2003. Release strategies of Amblyseius womersleyi and population dynamics of Amblyseius womersleyi and Tetranychus urticae: I. Release position in pear. J. Asia-Pacific Entomol. 6, 221–227.
- Helle, W., Sabelis, M.W., 1985. Spider Mites. Their Biology, Natural Enemies and Control A; World Crop Pests 1A. Elsevier, Amsterdam.

- Huffaker, C.B., Berryman, A., Turchin, P., 1999. Dynamics and regulation of insect populations. In: Huffaker, C.B., Gutierrez, A.P. (Eds.), Ecol. Entomol., 2nd ed. Wiley, New York, pp. 269–305.
- Hull, L.A., Asquith, D., Mowery, P.D., 1977. The mite searching ability of Stethorus punctum within an apple orchard. Environ. Entomol. 6, 684–688.
- Jung, C., 2005. Some evidences of aerial dispersal of twos-potted spider mite from an apple orchard into a soybean field. J. Asia-Pacific Entomol. 8, 279–283.
- Jung, C., Kim, D.-S., Park, Y.-S., Lee, J.-H., 2005. Simulation modeling of two spotted spider mite population dynamics in apple and pear orchards in Korea. J. Asia-Pacific Entomol. 8, 285–290.
- Kapur, A.P., 1948. On the old world species of the genus Stethorus Weise (Coleoptera: Coccinellidae). Bull. Entomol. Res. 39, 297–320.
- Kheradpir, N., Khalghani, J., Ostovan, H., Rezapanah, M.R., 2007. The comparison of demographic traits in *Tetranychus urticae* Koch (Acari: Tetranychidae) on five different greenhouse cucumber hybrids (*Cucumis sativus*). Acta Horti. 747, 425–430.
- Lactin, D.J., Holliday, N.J., Johnson, D.L., Craigen, R., 1995. Improved rate model of temperature-dependent development by arthropods. Environ. Entomol. 24, 68–73.
- Lamb, R.J., 1992. Developmental rate of Acyrthosiphon pisum (Homoptera: Aphididae) at low temperature: implications for estimating rate parameters for insects. Environ. Entomol. 21, 10–19.
- Lee, J.H., Elliott, N.C., 1998. Comparison of developmental response to temperature in *Aphelinus asychis* (Walker) from to different geographic regions. Southwest. Entomol. 23, 77–82.
- Logan, J.A., Wollkind, D.J., Hoyt, S.C., Tanigoshi, L.K., 1976. An analytical model for description of temperature dependent rate phenomena in arthropods. Environ. Entomol. 5, 1133–1140.
- McMurtry, J., Haffak, C.B., Devrie, M.V., 1970. Ecology of tetranychid enemies: their biological characters and impact of spray practices. Hilgardia. 40, 331–390.
- Medeiros, R.S., Ramalho, F.S., Serrao, J., Zanuncio, J.C., 2004. Estimative of *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) development time with non linear models. Neo. Entomol. 33, 141–148.
- Perdikis, D.C., Lykouressis, D.P., 2002. Thermal requirements for development of the polyphagous predator *Macrolophus pygmaeus* (Hemiptera: Miridae). Environ. Entomol. 31, 661–667.
- Putman, W.L., 1955. Bionomics of *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) in Ontario. Can. Entomol. 87, 9–33.
- Richardson, N.L. 1977. The biology of *Stethorus loxtoni* Britton and Lee (Coleoptera: Coccinellidae) and its potential as a predator of *Tetranychus urticae* Koch (Acari: Tetranychidae) in California. Ph.D. dissertation, University of California, Berkeley.
- Roy, M., Brodeur, J., Cloutier, C., 1999. Seasonal abundance of spider mites and their predators on red raspberry in Quebec. Environ. Entomol. 28, 735–747.
- Roy, M., Brodeur, J., Cloutier, C., 2002. Relationship between temperature and developmental rate of *Stethorus punctillum* (Coleoptera: Coccinellidae) and its prey *Tetranychus mcdanieli* (Acari: Tetranychidae). Environ. Entomol. 31, 177–187.
- Roy, M., Brodeur, J., Cloutier, C., 2005. Seasonal activity of the spider mite predators Stethorus punctillum (Coleoptera: Coccinellidae) and Neoseiulus fallacis (Acarina: Phytoseiidae) in raspberry, two predators of Tetranychus mcdanieli (Acarina: Tetranychidae). Biol. Control. 34, 47–57.
- Shih, C.I.T., Lin, P.J., Chang, T.W., 1991. Biology, predation, life table and intrinsic rate of increase of Stethorus loi Sasaji. Plant Prot. Bull. 33, 290–300.
- SPSS, 2004. SPSS Base 13.0 User's Guide. SPSS Incorporation, Chicago, IL.
- Wagner, T.L., Wu, H.I., Sharpe, P.J.H., Schoolfield, R.M., Coulson, R.N., 1984. Modeling insect developments rates: a literature review and application of a biophysical model. Ann. Entomol. Soc. Am. 77, 208–225.
- Worner, S.P., 1992. Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. Environ. Entomol. 21, 689–699.
- Yang, X., Shen, M., Xiong, J., Guo, Z., 1996. Approaches to enhance the effectiveness of biocontrol of *Panonychus citri* (Acari: Tetranychidae) with *Stethorus punctillum* (Coleoptera: Coccinellidae) in citrus orchards in Guizhou. Sys. Appl. Acarol. 1, 21–27.